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Changes of Appetite in Female Migratory Monarch Butterflies (*Danaus plexippus)*

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Senior Honors Thesis
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Spring 2001-2002

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For my Mom, Dad, Jason, John Austin, family, friends, and professors…

My research has been a time and life-consuming endeavor. Yet, I would not trade the sweat and sleepless days and nights for anything in the world. The invaluable lessons learned go far beyond the empirical data of the laboratory and the field. Hopefully, these lessons will stay with me for the rest of my life. I've learned...

...that the best lessons are learned at the feet of a mentor in Mother Nature's classroom;

...that I can walk a mile when I think I can't take another step;

...that sometimes all I need is a hand to hold and a heart to understand;

...that to ignore facts does not change facts;

...that the best way to learn is to surround myself with people smarter than I am;

...that life may be tough at times, but I am tougher;

...that the less time I have to work, the more organized I am and the more I get done;

...that happiness and growth come from climbing the mountain, not resting on the top of it.

I'm still climbing and learning!

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Abstract

This experiment examined the effects of adult feeding through time on the fat mass of female monarch butterflies in their fall migratory and overwintering periods. The goal of this was to determine if the appetite and fat storage ability of the migrating monarch butterfly change over the course of the fall migration and overwintering period. There were four different samples used in this experiment: Sweet Briar and Texas fall migrants and early and late Mexico overwintering butterflies. Each sample was divided into treatment groups that differed in diet (concentration of sucrose) and the number of days they were fed and kept alive. The results showed that the later season butterflies consumed less sucrose on a daily basis and that the ability to accumulate or maintain fat decreased from Sweet Briar to Texas to early Mexico overwintering to late Mexico overwintering butterflies. Furthermore, the data suggest that the conversion rate of sucrose to fat may decrease as the butterflies age. The results of this experiment have conservation and ecological implications because of the monarchs' dependency on their fat.

Introduction

Danaus plexippus, commonly called the Monarch Butterfly, is known for the long migration it makes each fall to its overwintering sites in California and in the mountains of central Mexico just south of the Tropic of Cancer. It is the only insect species known to migrate thousands of kilometers (Urquhart and Urquhart 1978, Brower 1985). To endure the 5-month overwintering period, the monarch is dependent upon the fat reserves it accumulates along the migration routes before its arrival at the overwintering sites.

Life Cycle

The life cycle of the Monarch begins when a mated female lays her eggs on a milkweed plant. When an egg hatches, a black, yellow and white-striped caterpillar is born. It feeds solely on various milkweed plant species. The caterpillar consumes all of the protein that it will need for its adult life (need citation). In addition to the protein, the caterpillar consumes other forms of nutrients that are necessary to maturation. It turns into a pupa, which further changes into an imago (adult Monarch). The female adult repeats the life cycle by mating and laying her eggs. The egg to adult maturation process takes about one month and the rate is determined by the photoperiod and temperature.

Overwintering and Migration

The generation of monarchs that inhabits the eastern United States and southern Canada from the late summer to early autumn starts to migrate south to Mexico, just south of the Tropic of Capricorn. The butterflies arrive in their overwintering sites in the high altitude mountains of central Mexico, just escaping the cold in late November as larval food and nectar sources dwindle in North America. Monarchs that overwinter are in a state of reproductive diapause. Their sexual organs do not mature and they are unable to reproduce while they are in this state. The butterflies remain mostly inactive during the winter; however, some of the butterflies forage for nectar and water and fly or glide within a colony (Urquhart and Urquhart 1978, Brower 1985, Masters et al 1988, Calvert and Lawton 1993, Alonso-Mejía et al 1997).

Abies religiosa, or the Oyamel fir, dominates the overwintering sites in Mexico. In some of the overwintering sites as many as 10 million butterflies per hectare congregate on the branches, needles, and trunks of the fir trees (Calvert and Brower 1986).

The butterflies begin mating and leaving their overwintering sites in mid February, migrating back to the southern United States. They lay their eggs on milkweed plants, and then die. Once the eggs hatch, the larvae exploit the milkweeds. This new generation of monarchs continues to spread throughout the United States and into southern Canada where their ancestors lived. In late summer and early autumn, the third and fourth generation butterflies begin to migrate back to the overwintering sites and repeat the entire process.

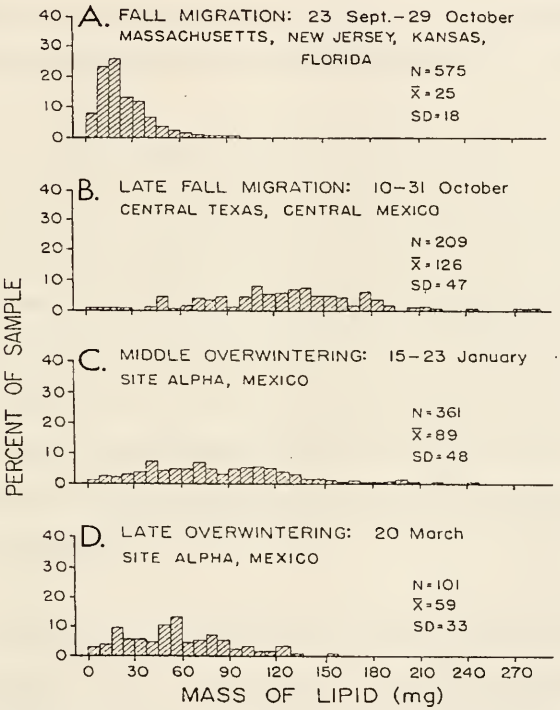
Butterfly Fat

The caterpillars and newly emerged adults acquire lipids by converting sugars into triglycerides (Cenedella 1971, Brown and Chippendale 1974, Turumen and Chippendale 1980, Gibo and McCurdy 1993). Since overwintering adults do not have access to abundant nectar resources in the high mountains of central Mexico during most of the winter, the fat that is acquired an a caterpillar and during the fall migration needs to provide energy to migrate to the Mexico, overwinter, mate, and migrate back to the southern United States. Bonds in fat molecules store 9 kcal of energy per gram and are the most efficient way to store energy because of their high mass-specific energy content (Kozhantshikov 1938, Beenakkers et al 1981, Picciano and Guthrie 1998).

The mass of lipids in a butterfly varies depending on where the monarch is in its fall migration and overwintering period. Walford (1980) and Brower (1985) found that the migratory generation of monarchs are

leanest during their initial migratory period; they stock up on fat reserves in the southern United States and northern Mexico before flying to their overwintering sites; and their fat reserves decrease as they use them to support life during their overwintering period (Figure 1).

Figure 1- Frequency Distribution of the fat mass of Monarch Butterflies during the fall migration and overwintering periods (Walford 1980 and Brower 1985)



Fat is not only necessary to provide energy for the overwintering period, it also plays an important role in monarch post diapause reproduction (Walford, 1980). The fertility of male insects remains mostly unaffected by the mass of their fat reserve, but the female's fertility is dependent on her fat reserve, because it is needed for vitellogenesis (Wigglesworth 1972, Evans 1984).

Conservation Concerns

The overwintering sites of the monarchs are being degraded by high rates of logging and clear cutting for agricultural purposes (Rzedowski 1983, Calvert et al 1989, Snook 1993, Brower et al 2002). Some monarchs,

therefore, spend their winters in areas that have been thinned (open forests). The closed forests that have not been thinned give the butterflies a more protected environment. Temperatures fluctuate more in the open areas, reaching higher and lower ambient temperatures than experienced in the closed areas (Brower 1995, Alonso-Mejía et al 1997). The open areas also have higher rates of evaporation and higher wind velocities. Alonso-Mejía et al (1997) hypothesized that butterflies roosting in the trees in closed areas had higher survivorship rates than those open areas and that the butterflies in the open forests had higher rates of lipid use. It has been demonstrated that butterflies that forage have less fat than the inactive butterflies in clusters (Brower and Malcolm 1991, Alonso-Mejía et al 1997).

Behavior

The spring and summer generations differ from the late summer and autumn generations physiologically and behaviorally. The late summer and fall butterflies are in a state of reproductive diapause, while the spring and summer butterflies form breeding populations. The migrant butterflies accumulate extensive lipid stores as they migrate south. In contrast, the spring and summer monarchs do not accumulate large lipid supplies (Beall 1948, Tuskes and Brower 1978, Walford 1980, James 1984, Brower 1985). The migrant generation of butterflies also displays behavioral changes over the course of the fall migration and overwintering period. During the fall migration the butterflies are nectaring and migrating south and in the winter the butterflies typically do not feed and remain mostly inactive. The behavioral changes that occur in the migratory butterfly might directly affect the butterfly's ability to accumulate fat.

Pilot Study

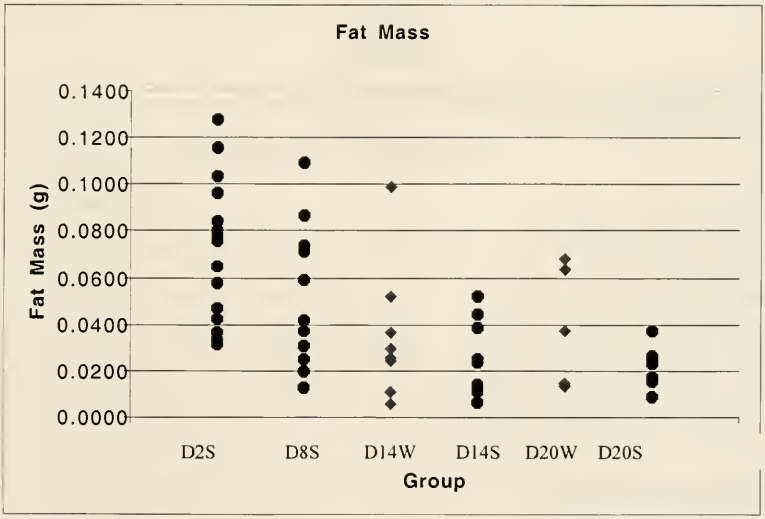
Last year I conducted a pilot experiment to examine the effects of diet on the fat mass of California late overwintering female *Danaus plexippus*. I wanted to know if overwintering female monarch butterflies could accumulate new fat reserves when allowed to feed on unlimited amounts of sucrose. In addition, I wanted to know how diet affects the fat mass of female butterflies through time: I hypothesized that butterflies receiving a sugar water solution would gain fat mass through time and that butterflies that only received water would lose fat mass through time. I tested my hypothesis by dividing my original sample size into groups that were killed off in pre-set increments. The groups differed in the number of days they were fed sucrose and water. A group of butterflies were killed on Day 2 and the remainder of the sample was fed of sucrose solution every other day through Day 8 when another group of butterflies was killed. The remaining butterflies were divided and fed either water or 20% sucrose

solution every other day, and killed on Day 14 or Day 20 (Table 1). There was a significant decrease in the fat masses of the all groups over time: the sugar water diet did not make the butterflies gain fat (Figure 2). There was also a significant difference between the fat mass of water fed butterflies and sugar water fed butterflies. Contrary to my prediction, however, the) groups had larger fat masses than the sugar water groups (D14S and D20S). The sugar water fed butterflies lost as much fat as, if not more than, the butterflies that were only fed water.

Table 1- Feeding Schedule used for a laboratory feeding experiment performed in spring of 2001 that examined the effects of diet on the fat mass of female California overwintering butterflies. The numbers represent the number of days the group was fed sugar water or plainwater.

GROUP	D2S	D8S	D14W	D14S	D20W	D20S
FEEDING SCHEDULE						
SUGAR $H_20 \rightarrow$	0	6	6	12	6	18
$H_20 \rightarrow$	0	0	6	0	12	0

Figure 2 - Fat mass of female monarch butterflies from a laboratory feeding experiment that was performed in the spring of 2001. (The letter "D" followed by the number represents the day that a group of butterflies was killed. The "S" represents butterflies that were fed a 20% sucrose solution and the "W" represents butterflies that were fed water after Day 8 of the experiment.



There are two different explanations for the results of last year's experiment: (1) overwintering female monarch butterflies cannot accumulate fat or, (2) the butterflies were not able to accumulate fat because of the experimental conditions.

Hypothesis

This year I continued my examination of the fat mass of female monarch butterflies. I want to know if the monarch's appetite and ability to accumulate fat changes over the course of the fall migration and overwintering period. In the fall, migrating butterflies are observed nectaring frequently to accumulate fat to provide them with energy for the overwintering period (Cenedella 1971, Brown and Chippendale 1974, Turunen and Chippendale 1980). In contrast, during the winter the butterflies are usually inactive. Active behaviors such as flight and nectaring would utilize the fat reserves more quickly (Masters, et al 1988) and it has been proposed that the inactivity is adaptive, to maintain fat reserves. If inactivity in the overwintering sites is adaptive, then decreased appetite could be favored by natural selection because the energetic cost of nectaring is probably more than the benefits of nectaring in the overwintering sites. To examine this hypothesis, I tested the consumption and fat accumulation of Sweet Briar and Texas fall migrants and early and late Mexico overwintering butterflies when maintained under standard laboratory conditions. I hypothesized that the fall migrants would consume more sucrose and accumulate fat over the duration of the experiment and the overwintering butterflies would consume less sucrose solution and lose fat over the duration of the experiment.

Subjects

The subjects of this experiment were female monarch butterflies from the eastern population collected alive during four different parts of the fall migration and overwintering period.

Sweet Briar Fall Migrants (SBC)

The first group of butterflies was caught on Sweet Briar College campus at the Train Station, Guion, and Meta Glass butterfly bushes (*Buddleia davidii*) between the 17th of September and the 1st of October of 2001 (N=140). As the butterflies were captured, they were placed in a cooler with ice and a moist paper towel to prevent the monarchs from drying out overnight. Inside the cooler, the monarchs were held individually in glassine envelopes. They were held in the cooler overnight to replicate the conditions of the butterflies in subsequent samples that had to be shipped from Texas and Mexico. The next day, the butterflies were place into the environmental chamber.

Texas Fall Migrants (TX)

The second group of butterflies was shipped via Federal Express overnight in two different shipments from Eagle Pass, TX. As above, the butterflies were packaged in individual envelopes in a cooler with freezer packs and moist paper towels. The first batch (N= 57) was collected and shipped on the 9th of October and arrived at Sweet Briar on the 10th. The second batch (N= 43) was collected and shipped on the 16th and arrived on the 17th. On the day that the butterflies arrived, I placed them into the environmental chamber.

Early Mexico Overwintering (MX1)

The early Mexico overwintering butterflies (N= 50) were captured on the 11th of January 2002. These butterflies were taken from a roost in the center of the Zapatero overwintering colony in the Sierra Chincua in Michoacán. They were shipped in a cooler on freezer packs and arrived in the laboratory on the 15th of January. They were placed into the environmental chamber the day that they arrived.

Late Mexico Overwintering (MX2)

I collected the late Mexico overwintering butterflies (MX2) (N= 60) on the 6th of March 2002. The butterflies were taken from a single roosting cluster on the branches of an Oyamel fir (*Abies religiosa*) approximately 7.8m off of the ground in the Zapatero colony in Sierra Chincua. A net was attached with duct tape to a pole made from a small dead fir tree. The total length of the net and the pole was 6.75m. The butterflies were placed individually in glassine envelopes in a cooler with ice and flown home with me on the 7th of March. On the 8th of March, the butterflies were placed into the environmental chamber.

Methods

Experimental Schedule

The basic time line of my experiment began with Day 0 when all of the butterflies were weighed (Mettler AT250C) and a subgroup of butterflies was frozen, and the remaining monarchs were divided into groups to be fed the 20% or 45% sucrose solution (the SBC group also included butterflies that were fed 35% sucrose). The SBC and TX butterflies were further divided into groups that would be killed by freezing on Days 7 and 14. The MX1 and MX2 overwintering butterflies only contained groups of butterflies that would be killed on Day 7 (Table 2). Individual butterflies were assigned to groups using a random number table.

Table 2- Treatment Groups and Sample Sizes
SBC= Sweet Briar Fall Migrants, TX= Texas Fall Migrants, MX1= Early Mexico Overwintering, and MX2= Late Mexico Overwintering
The "✕" indicates that treatment was not done in the group of butterflies.

GROUP NAME	DIET	NUMBER OF DAYS KEPT ALIVE	NUMBER OF TIMES FED	SAMPLE SIZES			
				SBC	TX	MX1	MX2
Day 0	Not fed	0	0	19	19	18	20
20% Day 7	20% sucrose	7	7	19	22	15	20
35% Day 7	35% sucrose	7	7	18	✕	✕	✕
45% Day 7	45% sucrose	7	7	17	21	16	20
20% Day 14	20% sucrose	14	14	19	18	✕	✕
35% Day 14	35% sucrose	14	14	21	✕	✕	✕
45% Day 14	45% sucrose	14	14	22	19	✕	✕

The sample sizes of my treatment groups varied because some of the butterflies died prematurely. The sample size numbers in Table 1 represent the total number of butterflies that remained alive until they reached the end of their experimental treatment and were frozen.

Environmental Chamber Conditions

Butterflies were kept in an EGC (model G10) (Environmental Growth Chambers, Chagrin Falls, Ohio). Inside the chamber, the butterflies were exposed to a 12:12 LD fluorescent light regime each day. The chamber was set at a constant temperature of 25°C and relative humidity of 75%. In previous experiments in these environmental chambers there were problems with the butterflies drying out. To prevent this, I kept the butterflies in glassine envelopes inside a clear plastic shoebox containing a finger bowl of oxalic acid. At 25°C the acid buffers the air so that it is 75% humidity (Weast and Shelby, 1967). The saturated oxalic acid solution was prepared by putting enough acid crystals into a glass finger bowl to cover the bottom of the dish. Then, approximately 20ml of distilled water was added to the bowl. About 30 to 40 butterflies in their individual envelopes were placed in each plastic box, making sure that they did not directly contact the oxalic acid solution.

I changed the envelopes that housed the butterflies at least every three days to prevent them from becoming fouled in their waste products. Additional distilled water was added to the finger bowls that contained the oxalic acid as needed.

Butterfly Feeding

Each day the butterflies were removed from the environmental chamber and fed. I measured the amount the butterflies ate by using a device informally known as a 'suck-o-meter' (May, 1985). The suck-o-meter was prepared by taping a metric ruler to the sloped (15°) side of a piece of wood resembling a doorstop. I next filled a 100_l Van-Lab capillary tube to its calibrated line with the designated solution of sucrose (20 %, 35%, or 45%). The bottom of the capillary tube was lined up with the zero end of the ruler. I prepared up to 20 suck-o-meters at a time to speed up the feeding process. Each capillary tube was used in only one feeding session.

I held a butterfly by the base of its wings and enticed it to drink by simply inserting an insect pin into the center loop of its proboscis and carefully stretching it towards the bottom of the tube. If the proboscis needed extra guidance, I would poke the tip of the pin into the pipette tube while the extended proboscis was wrapped around it. I placed small weights on the butterflies' wings so that I could direct my attention elsewhere or if they were being 'obstreperous' and trying to fly away. If a butterfly finished a tube of solution, I moved it to a new tube until it did not eat any more. I recorded the number of whole tubes each butterfly consumed and the amount that was remaining in any unfinished tubes to the nearest 0.1mm. I encouraged each butterfly to drink several times before ending a session and placing it back into its envelope. If I could not get a butterfly to consume any food, I would put it away and try again after I had finished feeding the rest of the butterflies. After each insect had finished drinking, I would wash its legs and proboscis by dipping them into a water-filled petri dish to prevent bacteria from thriving.

Butterfly Food

The butterfly food was prepared by adding the appropriate amount of sucrose (table sugar) to a flask and then adding distilled water to the 100.0ml mark (Table 3). The sugar solution was stored in capped bottles and refrigerated. Before feeding the butterflies, the sugar water was allowed to come to room temperature. I made a new solution of sugar water and changed the water every three days to lower the risk of bacterial contamination.

Table 3- Amount of Sucrose Added to Make 100ml of the 20%, 35%, and 45% Solutions and Conversion Factors used for Calculating Grams of Sucrose Consumed

CONCENTRATION	GRAMS OF SUCROSE	CONVERSION FACTOR (g/µl)
20%	20.00	2.0×10^{-4}
35%	35.00	3.5×10^{-4}
45%	45.00	4.5×10^{-4}

Feeding and Consumption *Calculations*

The amount each butterfly consumed was recorded each day to the nearest 0.1 mm. The total length of a 100µl Van-Lab capillary tube was 75.0mm; therefore, the conversion factor between millimeters and micro liters is 1.33µl / mm.

The total volume consumed from Day 0 to 6 (week 1) was calculated for each butterfly, and the volume consumed from Day 7 to 13 (week 2) and the total volume consumed from Day 0 –13 was calculated for the butterflies that were killed on Day 14. The mass of sucrose consumed in each time interval was calculated by multiplying the volume consumed (_l) by the appropriate conversion factor (Table 3).

Size Measurements

I measured the forewing lengths of the frozen butterflies to the nearest 0.1 millimeter based on the distance from the base of the right forewing where it attaches to the thorax to the apex of the wing (Van Hook 1993). Measured monarchs were placed into new glassine envelopes. I marked the butterflies' number on each envelope with a permanent pen. Then, several holes were punched into each envelope with a hole-puncher. Next, the butterflies were placed in a Thelco drying oven, where they were dried for 16 hours at 60°C. This process rid their bodies of any existing water (Cenedella 1971).

Dried butterflies were weighed (Mettler AT250C) to the nearest 0.0001 grams and placed in a numbered, 35ml Kimax screw cap centrifuge tubes. I put the butterflies into the tubes headfirst so that their brittle legs and antennae would break off into the bottle. (If any body parts fell outside of the bottle, I made certain to gather them up and put them in the bottle.) I kept track of the butterflies' identification numbers and the tubes in which they were placed. I stored the dry butterflies in the tubes with the tops on until I could perform the lipid analysis.

Lipid Analysis

I followed the methods described in Walford (1980) as modified by Alonso-Mejia et al (1997). To extract the lipids, I first added 10ml of petroleum ether to each screw top centrifuge tube that contained a butterfly. A glass rod was used to crush the butterfly's wing pieces in the bottom of the tube so that the petroleum ether was covering them. I kept the tubes capped until it was time to grind them up.

I finely ground each individual butterfly body and wings, using an Ulta-Turrax tissuemizer for approximately 2 minutes. To avoid overheating the apparatus, each butterfly was ground in 30-second increments. The centrifuge tubes were worked up and down on the tissuemizer post, but I never allowed the post to come completely out of the petroleum ether solution. Some of the butterfly material adhered to the sides of the post, the amount varying with each butterfly. I allowed the material to dry slightly and then I pipetted a small amount of ether on the side to rinse the parts back into the bottle. The slightly dried butterfly material made the post easier to clean; however, it was not advisable to let the material become too dry, because the butterfly pieces can blow away. I next put 5ml of petroleum ether into a Corex test tube and rinsed off the tissuemizer post and added the contents from the tube to the centrifuge tube. The cap was then screwed securely on the centrifuge tube. The centrifuge tube was filled no higher than _ of the way to the diamond marker on the side of the tube (the vortex mixer and aluminum weighing pans cannot handle any more than this). I wiped the outside of the post and cleaned it off between each butterfly with 1 soapy water tube, 3 distilled water tubes, 2 alcohol tubes, and 2 petroleum ether tubes. The tubes were moved up and down in the same fashion as grinding. The cleansing tubes were never used more than once.

I vortexed the tube for 10 seconds with a Thermolyne vortex and placed it in a Precision shaker bath at a constant temperature of 35°C and at a speed of 85RPM for 30 minutes. The tubes were removed from the bath and vortexed every 10 minutes for 10 seconds.

At the end of the 30 minutes, the tubes were centrifuged in a DYNAC centrifuge for 10 minutes with the speed set at 30. This separated the contents into two layers. The bottom layer (solids) was the butterfly's lean mass. The top layer (liquids) was the butterfly's fat dissolved in the petroleum ether. The top layer was carefully pipetted off using a pasteur pipette and placed in pre-weighed aluminum tins. A different pasteur pipette was used for each butterfly and each butterfly had its own set of aluminum tins (2) stacked inside of each other. A pair of forceps was used to gently etch a butterfly's number and bottle number inside of the top tin. The tins were placed on a C. S. &

E. slide warmer set at 30°C (setting 4) to help evaporate the petroleum ether under a hood with the fan on. When I finished taking the top layer off, I added 20ml of petroleum ether back into the centrifuge tube and repeated the shaker bath, centrifugation, and extraction procedures. After the second run, I washed the remaining butterfly parts down the drain. The ether was allowed to evaporate from the tins overnight. The mass of the tins plus the fat was taken after all of the ether was gone.

Lipid Calculations

The mass of the dried butterfly, the mass of the set of tins, and the mass of the fat and the set of tins were recorded to the nearest 0.0001 grams. This enabled me to calculate each butterfly's fat mass and lean weight. The mass of the fat was calculated by subtracting the mass of the set of tins from the mass of the tins plus fat. The mass of the fat was then subtracted from the original dry weight of the butterfly to give its lean weight.

Statistical Analyses

The lean weight, wing length, and initial wet weight data from each group of butterflies were compared with a 1-Factor ANOVA. If the ANOVA was significant the data were further analyzed with a Fisher's Post hoc Least Significant Difference test to determine which samples differed. The final fat mass of butterflies fed for 0, 7, or 14 days were compared with a 1-Factor ANOVA for each sucrose concentration for each sample. Combined effects of feeding duration and combined sucrose concentration on the fat mass of the SBC and TX butterflies that were fed for 7 or 14 days were analyzed with a 2-Factor ANOVA with number of days fed and concentration of sucrose as main effects. Two-Factor ANOVA was also used to compare consumption of the SBC, TX, MX1, and MX2 butterflies. A paired T-test was used to reveal any significant differences in the volume consumed from Days 0 –6 verses Days 7 –13 for the SBC and TX butterflies that were killed on Day 14.

Results

SBC, TX, MX1, and MX2 Day 0 Butterflies

Standard Size Measurements. Since the wing length and lean weight do not change after an adult ecloses, the samples of butterflies should not vary in these measures (Walford 1980). The Day 0 butterflies from SBC, TX, MX1 and MX2, killed on the 1st day of the experiment did not differ in their wing lengths (1-Factor ANOVA, $F_{3, 72}$ = 2.28, p= 0.087). However, there was a significant difference in their lean weights (1-Factor ANOVA, $F_{3, 72}$ = 2.77,

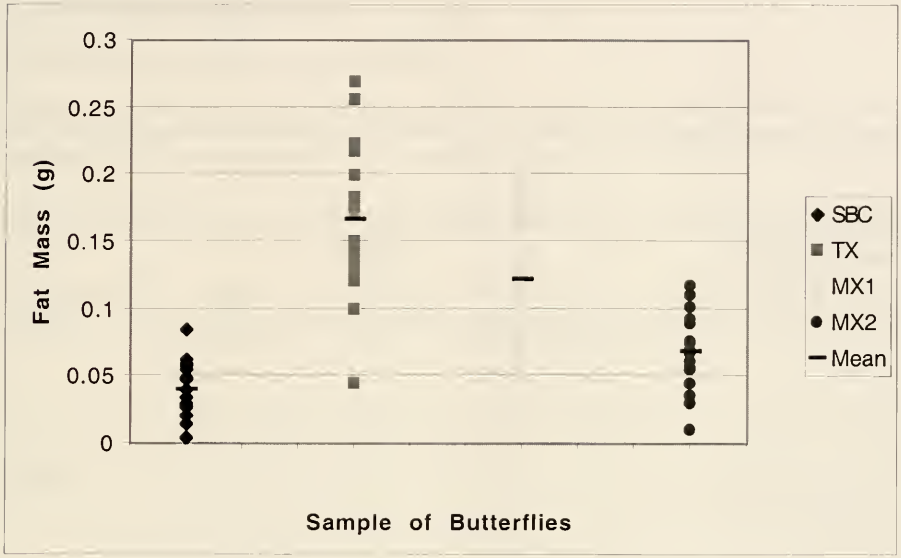
p= 0.048). The TX butterflies had significantly higher lean weights than the MX1, but none of the other groups differed significantly from one another (Table 4).

Fat. There were significant differences in the fat mass (Figure 3) 1-Factor ANOVA, $F_{3, 72} = 35.60$, $p < 0.0001$) and the dry weight (1-Factor ANOVA, $F_{3, 72} = 23.16$, $p<0.0001$) of the Day 0 butterflies from the four samples. The SBC monarchs were the leanest group and had a mean fat mass of 0.037g. The TX butterflies had the highest mean fat mass (0.166g). Each of the four samples differed significantly from each of the other three ($p =$ or < 0.0001 for all pairwise comparisons).

Table 4- Day 0 Butterflies' Mean Wing Length, Lean Weight, Fat Mass, and Dry Weight

GROUP	WING LENGTH (mm)		LEAN WEIGHT (g)		FAT MASS (g)		DRY WEIGHT (g)	
	Mean	Stdev	Mean	Stdev	Mean.	Stdev.	Mean	Stdev.
SBC	49.7	1.87	0.163	0.018	0.037	0.019	0.207	0.007
TX	51.4	2.10	0.182	0.034	0.166	0.055	0.348	0.017
MX1	50.8	3.08	0.153	0.036	0.122	0.053	0.275	0.017
MX2	49.8	2.43	0.165	0.032	0.068	0.027	0.233	0.008

Figure 3-Fat Mass of Day 0 Butterflies From All Four Samples



Sweet Briar Fall Migrants

The SBC butterflies consumed more sucrose solution than the other samples of butterflies. The butterflies that were fed for 1 or 2 weeks put on fat with the 20%, 35%, and 45% sucrose solutions.

Standard Size Measurements. There were no significant differences in the wing length, initial wet weight, and lean weight of the treatment groups of SBC Fall migrants (Table 5).

Table 5- 1-Factor ANOVAs for Size Measurements of Sweet Briar Butterflies (N.S.= not significant)

MEASUREMENT	DEGREES OF FREEDOM	F-VALUE	P-VALUE
WING LENGTH	11, 133	1.67	N.S.
INITIAL WET WEIGHT	11, 133	1.18	N.S.
LEAN WEIGHT	11, 133	0.60	N.S.

Sucrose Consumption. For each time interval (Days 0 –6, Days 7 –13, Days 0 –13), there was no significant difference in the volumes of sucrose solution consumed by butterflies fed the 20%, 35%, or 45% sucrose

solutions (Figure 4 and Table 6). Consequently, the butterflies that were fed the higher concentration of sucrose solution consumed significantly more grams of sucrose than the butterflies that were fed the lower concentrations of sucrose for Day 0 –6 and Day 7 –13 (Figure 5 and Table 6).

Table 6-1 Factor ANOVA Comparing Volumes of Solution and Grams of Sucrose Consumed by the 20%, 35%, and 45% Sweet Briar Butterflies (For the Day 7 –13 and Day 0 –13 the data from the butterflies that were killed on Day 7 were not included.)(N.S.= not significant, *<0.05, and * <0.0001)**

MEASUREMENT	PERIOD	DEGREES OF FREEDOM	F-VALUE	P-VALUE	PAIRWISE COMPARISONS FOR CONCENTRATION (P-VALUE)		
					20 vs. 35	20 vs. 45	35 vs. 45
VOLUME	DAY 0 –6	2, 111	1.18	N.S.			
	DAY 7 –13	2, 57	2.78	N.S.			
	DAY 0 –13	2, 57	2.10	N.S.			
GRAMS	DAY 0 –6	2, 111	99.83	***	***	***	***
	DAY 7 –13	2, 57	20.65	***	***	***	**
	DAY 0 –13	2,57	42.31	***	***	***	***

Fat. For each sucrose concentration butterflies that were fed for 1 and 2 weeks had significantly higher fat masses than the Day 0 butterflies (Figure 6 And Table 7). At the 45% concentration, the butterflies that were fed for 2 weeks had significantly higher fat masses than the butterflies that were fed for 1 week. In addition, the concentration of sucrose had a significant effect on the butterflies' fat mass at death: the 35% and 45% sucrose fed butterflies had significantly higher fat masses than the 20% sucrose fed butterflies (Table 8).

Table 7- 1 Factor ANOVA of Final Fat Mass of Sweet Briar Butterflies, Comparing Butterflies That Were Fed for 0, 7, or 14 Days for Each Sucrose Concentration (Fisher's PLSD was used for Individual Comparisons)(N.S.= not significant, *<0.05, and *** <0.0001))

DIET	DEGREES OF FREEDOM	F-VALUE	P-VALUE	PAIRWISE COMPARISONS FOR FAT (P-VALUE)		
				0 vs. 7	0 vs. 14	7 vs. 14
20%	2,55	27.421	***	***	***	N.S.
35%	2,55	33.874	***	***	***	N.S.
45%	2,55	68.833	***	***	***	*

Table 8- 2 Factor ANOVA Comparing the Effects of Sucrose Concentration (20%, 35%, or 45%) and Time (one week vs. two week) on final fat mass of the Sweet Briar Butterflies (Fisher's PLSD was used for Individual Comparisons) (N.S.=not significant and ***<0.001)

	DEGREES OF FREEDOM	F-VALUE	P-VALUE	PAIRWISE COMPARISIONS FOR CONCENTRATION (P-VALUE)		
				20 vs. 35	20 vs. 45	35 vs. 45
CONCENTRATION	2	17.56	***	***	***	N.S.
TIME	1	3.40	N.S.			
CONCENTRATION * TIME	2	1.27	N.S.			
RESIDUAL	110					

Texas Fall Migrants

The TX butterflies consumed the second highest amount of sucrose solution. The 45% fed butterflies accumulated fat and the 20% butterflies maintained their fat.

Standard Size Measurements. There were no significant differences among groups in wing length, initial wet weight, and lean weight (Table 9).

Table 9- 1 Factor ANOVAs for Size Measurements of Texas Butterflies (N.S.=not significant)

MEASUREMENT	DEGREES OF FREEDOM	F-VALUE	P-VALUE
WING LENGTH	4, 56	4.56	N.S.
INITIAL WET WEIGHT	4, 56	0.463	N.S.
LEAN WEIGHT	4, 56	0.216	N.S.

Sucrose Consumption. For each time interval (Days 0 –6, Days 7 –13, and Days 1 –13) the 20% sucrose fed butterflies consumed a significantly greater volume of solution than the 45% sucrose fed butterflies (Figure 7 and Table 10). However, the 45% fed butterflies consumed significantly more grams of sucrose than the 20% fed butterflies (Figure 8 and Table 10). Consumption decreased from the first week to the second week in both the 20% (Paired T-test, T_{17}= 5.122, P <0.0001) and 45% (Paired T-test, T_{17}= 5.225, P <0.0001) sucrose fed butterflies.

Table 10- 1 Factor ANOVAs Comparing Volume and Grams of Sucrose Consumed by the 20% and 45% Texas Butterflies (For the Day 7 –13 and Day 0 –13 the data from the butterflies that were killed on Day 7 were not included.) (N.S.=not significant, *<0.05, **<0.005 and ***<0.001)

MEASURMENT	DAYS	DEGREES OF FREEDOM	F-VALUE	P-VALUE
VOLUME	DAY 0 –6	6, 107	14.84	**
	DAY 7 –13	1, 34	7.98	*
	DAY 0 –13	1, 34	7.57	*
GRAMS	DAY 0 –6	1, 78	77.60	***
	DAY 7 –13	1, 35	40.23	***
	DAY 0 –13	1,35	77.60	***

Fat. The 20% fed butterflies maintained their fat mass over the duration of the experiment and the 45% sucrose fed butterflies increased their fat over the duration of the experiment. The 45% sucrose fed butterflies had significantly higher fat masses than the Day 0 butterflies. On the other hand, there were no significant differences between the 20% sucrose fed butterflies and the butterflies that were killed on Day 0 (Figure 9 and Table 11). There

was no significant difference in the fat mass of butterflies that were killed on Day 7 verses Day 14. However, the fat mass of the butterflies was significantly affected by the concentration of sucrose they were fed. The number of days they were kept alive had no significant affect on the final fat mass (Figure 9 and Table 12).

Table 11- 1 Factor ANOVAs Comparing Final Fat Mass of Texas Butterflies That Were Killed on Day 0, 7, and 14 (Fisher's PLSD was used for Individual Comparisons) (N.S.= no significant difference) (N.S.=not significant, *<0.05, **<0.005 and ***<0.001)

DIET	DEGREES OF FREEDOM	F-VALUE	P-VALUE	PAIRWISE COMPARISONS FOR FAT (P-VALUE)		
				0 vs. 7	0 vs. 14	7 vs. 14
20%	2,58	0.15	N.S.	N.S.	N.S.	N.S.
45%	2,58	5.71	*	**	*	N.S.

Table 12- 2 Factor ANOVA Comparing the Effects of Sucrose Concentration (20% or 45%) and Time on the Fat Mass of Texas Butterflies (Fisher's PLSD was used for Individual Comparisons) (N.S.=not significant and ***<0.001)

	DEGREES OF FREEDOM	F-VALUE	P-VALUE
CONCENTRATION	1	21.43	***
TIME	1	0.38	N.S.
CONCENTRATION * TIME	1	0.04	N.S.
RESIDUAL	78		

Early Mexico Overwintering

The MX1 overwintering butterflies consumed less sucrose solution than the SBC and TX butterflies. The 45% fed butterflies maintained their fat mass over the duration of the experiment and the 20% fed butterflies lost fat over the duration of the experiment.

Standard Size Measurements. There were no significant differences in the wing length, initial wet weight, and lean weight of the Early Mexico Overwintering butterflies (Table 13).

Table 13- 1-Factor ANOVAs for Size Measurements of Early Mexico Overwintering (N.S.=not significant)

MEASUREMENT	DEGREES OF FREEDOM	F-VALUE	P-VALUE
WING LENGTH	1, 29	0.196	N.S.
INITIAL WET WEIGHT	1, 29	0.330	N.S.
LEAN WEIGHT	1, 29	0.001	N.S.

Sucrose Consumption. The 20% fed butterflies consumed a significantly greater volume of solution than the 45% fed butterflies (Figure 10, 1-Factor ANOVA, $F_{1, 29} = 4.95$, $p = 0.034$). However, the 45% fed butterflies consumed significantly greater amounts of sucrose than the 20% fed butterflies (Figure 11, 1-Factor ANOVA, $F_{1, 29} = 22.76$, $p = <0.0001$).

Fat. There were no significant differences in the fat mass of the 20% and 45% fed butterflies (Figure 12, 1-Factor ANOVA, $F_{1, 29} = 2.856$, $p = 0.1017$). The 20% sucrose fed butterflies had significantly less fat than the Day 0 butterflies, while there was no significant difference between the 45% fed butterflies and the Day 0 butterflies (Table 14 and Figure 12).

Table 14- 1 -Factor ANOVAs Comparing the Fat Mass of the Early Mexico Overwintering Butterflies That Were Killed on Day 0 and 7 (N.S.=not significant and *<0.05)

DIET	DEGREES OF FREEDOM	F-VALUE	P-VALUE
20%	1, 31	4.31	*
45%	1, 33	0.22	N.S.

Late Mexico Overwintering

The MX2 butterflies consumed less sucrose solution than the SBC and TX butterflies. Both the 20% and 45% fed butterflies lost fat over the duration of the experiment.

Standard Size Measurements. There was a significant difference in the wing lengths of the MX2 butterflies (Table 15). The 45% and the 20% sucrose fed butterflies had significantly larger wing lengths than the

Day 0 butterflies. There was no significant difference in the lean weights or initial wet weights of the MX2 overwintering butterflies.

Table 15- 1 Factor ANOVAs for Size Measurements of Late Mexico Overwintering (N.S.=not significant and **<0.005)

MEASUREMENT	DEGREES OF FREEDOM	F-VALUE	P-VALUE
WING LENGTH	2, 57	6.01	**
INITIAL WET WEIGHT	2, 57	0.89	N.S.
LEAN WEIGHT	2, 57	0.21	N.S.

Sucrose Consumption. There was no significant difference in the total volume consumed by the 20% and 45% sucrose fed butterflies (Figure 13, 1-Factor ANOVA, $F_{1, 37} = 3.091$, p= 0.0870). Therefore, the 45% sucrose fed butterflies consumed significantly more grams of sucrose than the 20% sucrose fed butterflies (Figure 14, 1-Factor ANOVA, $F_{1, 37} = 50.983$, p <0.0001).

Fat. There were no significant differences in the fat masses of the 20 % and 45% sucrose fed butterflies (Figure 15, 1-Factor ANOVA, $F_{1, 36} = 0.732$, p= 0.3977). Both the 20% and the 45% fed butterflies had significantly less fat than the Day 0 butterflies (Table 16).

Table 16- 1 Factor ANOVAs Comparing the Fat Mass of Late Mexico Overwintering Butterflies That Were Killed on Day 0 and 7 (*<0.05 and **<0.005)

DIET	DEGREES OF FREEDOM	F-VALUE	P-VALUE
20%	1, 36	11.76	**
45%	1, 36	5.08	*

Comparison of the Four Samples: Consumption

Volume. The butterflies from the four samples responded differently to the provided food. The SBC butterflies consumed the most solution, the TX butterflies consumed the second highest amount of solution, and the

MX1 and MX2 butterflies consumed the least amount of solution (Figure 16). To compare the consumption data of the four samples of butterflies I ran a 2 Factor ANOVA on the data for butterflies fed 20% or 45% sucrose for 1 week. Both sucrose concentration and samples had significant effects on consumption (Table 17). Each of the 4 samples differed from all others except for the MX1 versus MX2.

Grams. The butterflies from the four samples consumed different amounts of sucrose. The SBC butterflies consumed the most sucrose, the TX butterflies consumed the second highest amount of sucrose, and the MX1 and MX2 butterflies consumed the least amount of sucrose (Figure 17). To compare the mass of sucrose consumed by the different samples I ran a 2-Factor ANOVA on the data for the 20% or 45% sucrose for 1 week (Table 18). Both the sucrose concentration and the samples had significant effects on the grams of sucrose consumed. All of the samples differed significantly except the MX1 versus MX2 butterflies. The significant interaction between the concentration of sucrose and sample of butterflies means that the butterflies from the 4 samples did not respond in the same way to the low versus high sucrose concentration. The difference between the amount of sucrose consumed between the high versus low sucrose fed butterflies was greater for the SBC butterflies than the TX, MX1, and MX2 butterflies.

Figure 16- Comparison of the total volume consumed consumed from day 0 –6 for the four samples

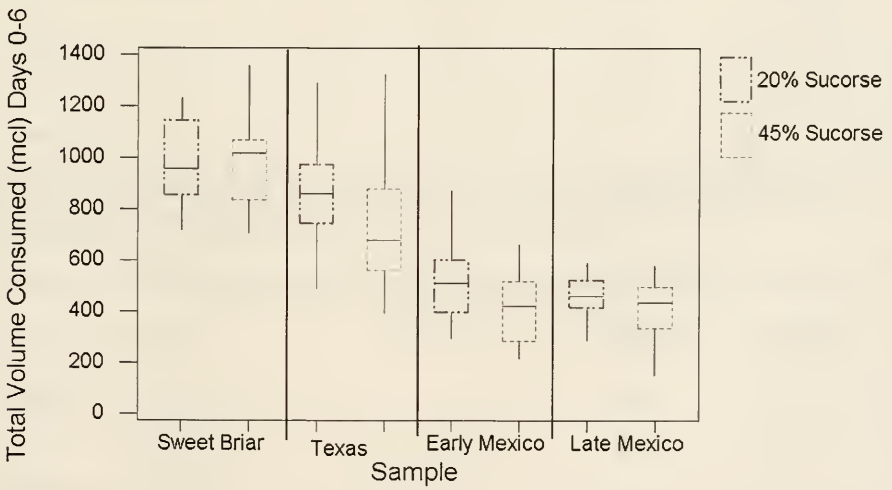


Figure 17- Comparison of the total mass of sucrose from day 0 –6 for the four samples

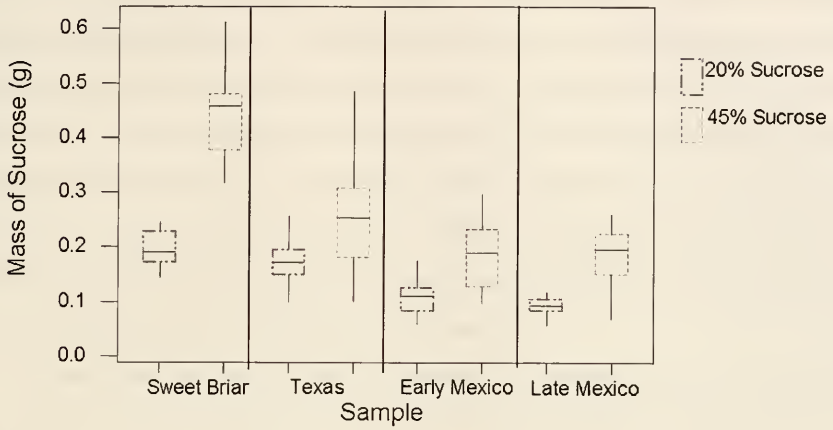


Table 17- 2- Factor ANOVA Results, Overview of Consumption Volume for Sweet Briar and Texas Fall Migrants and Early and Late Mexico Overwintering Butterflies (Table uses data from butterflies that were fed 20% or 45% sucrose solution that were killed on Day 7) (N.S.=not significant, **<0.005 and ***<0.001)

	DEGREES OF FREEDOM	F-VALUE	P-VALUE
SUCROSE CONCENTRATION	1	12.21	**
SAMPLE	3	87.13	***
SUCROSE CONCENTRATION X SAMPLE	3	1.60	N.S.
RESIDUAL	141		

Table 18- 2- Factor ANOVA Results, Overview of Consumption (Grams of Sucrose) for Sweet Briar and Texas Fall Migrants and Early and Late Mexico Overwintering Butterflies (Table on uses data from butterflies that were fed 20% or 45% sucrose solution that were killed on Day 7) (***<0.001)

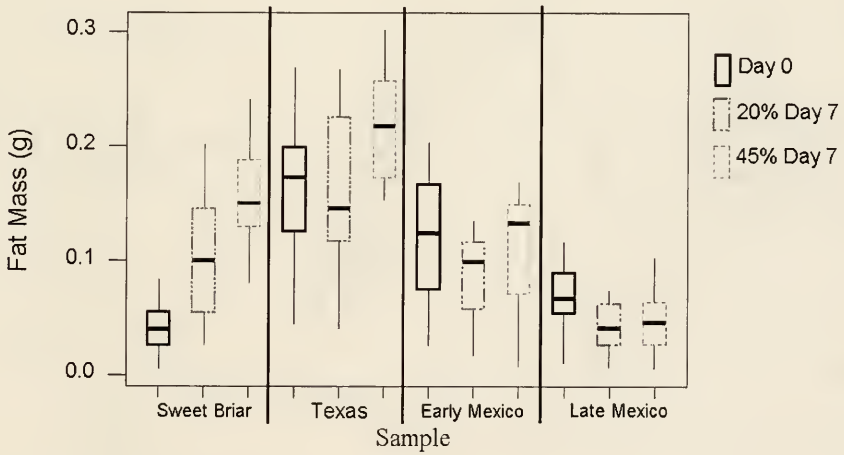
	DEGREES OF FREEDOM	F-VALUE	P-VALUE
SUCROSE CONCENTRATION	3	71.22	***
SAMPLE	1	182.21	***
SUCROSE CONCENTRATION X SAMPLE	3	12.17	***
RESIDUAL	141		

Comparison of the Four Samples: Final Fat

The final fat mass of the butterflies in each of the four samples was affected by sucrose concentration and the sample of butterflies (Figure 18). For the SBC sample, butterflies fed sucrose had significantly higher fat masses than the Day 0 butterflies and the butterflies that fed for two weeks had significantly higher fat masses than butterflies fed for 1 week. For the TX sample, butterflies that were fed the 45% sucrose had significantly higher fat masses than the 20% sucrose fed butterflies and the Day 0 butterflies. For the MX1 sample, the butterflies that were fed the 20% sucrose had significantly less fat than the Day 0 butterflies, while there was no significant difference in the fat mass of the 45% sucrose fed butterflies and the Day 0 butterflies. For the MX2 sample, the 20% and 45% sucrose fed butterflies had significantly less fat than the Day 0 butterflies.

I wanted to know how efficient each sample of butterflies was in converting sucrose to fat. Because initial fat masses varied, I could not just compare the final fat of the butterflies to the amount of sucrose they consumed.

Figure 18- Comparison of the final fat mass of the SBC, TX, MX1, and MX2 samples (x-axis: sample of butterflies and y-axis: fat mass (g))



Estimation of Initial Fat of Butterflies Killed on Day 7 or 14

A simple linear regression was used to plot the initial fat mass and initial wet weight of the Day 0 butterflies from each sample (Table 19 and Figure 19). The correlation coefficients were significant for the SBC, TX, and MX1 butterflies, but not for the MX2 butterflies. By inserting a butterfly's intial wet weight into the

regression equation, I obtained an estimate of its initial fat mass. For the SBC sample for example, a butterfly with an initial wet weight of 0.5587g would have an estimated fat mass of [y=0.2654(0.5587) – 0.0837= 0.0469]. For the SBC, TX, and MX1 samples, the initial wet weight of the butterflies that were killed on Day 7 or 14 was plugged into the regression equation determined for their group to give an estimate of their initial fat mass. The estimates of the initial fat mass were then used to calculate the fat gained or lost during the feeding ($Fat_{final} - Fat_{initial} = Fat_{gained}$).

Figure 19- Correlation Between Initial Wet Weight and Fat for Day 0 Butterflies From All Four Samples

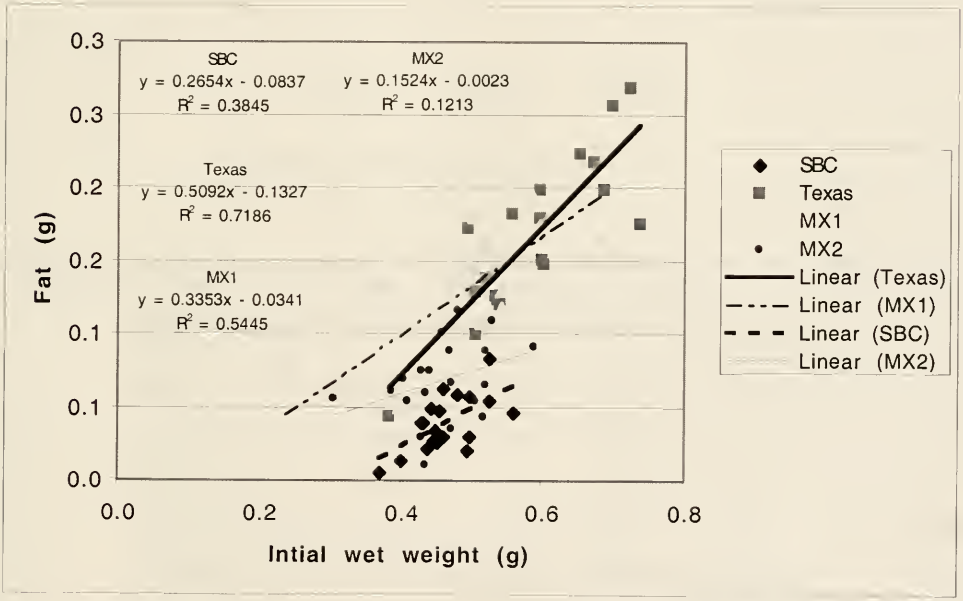


Figure 20- Efficiency (SBC, TX, MX1, and MX2) (Sucrose to Fat)

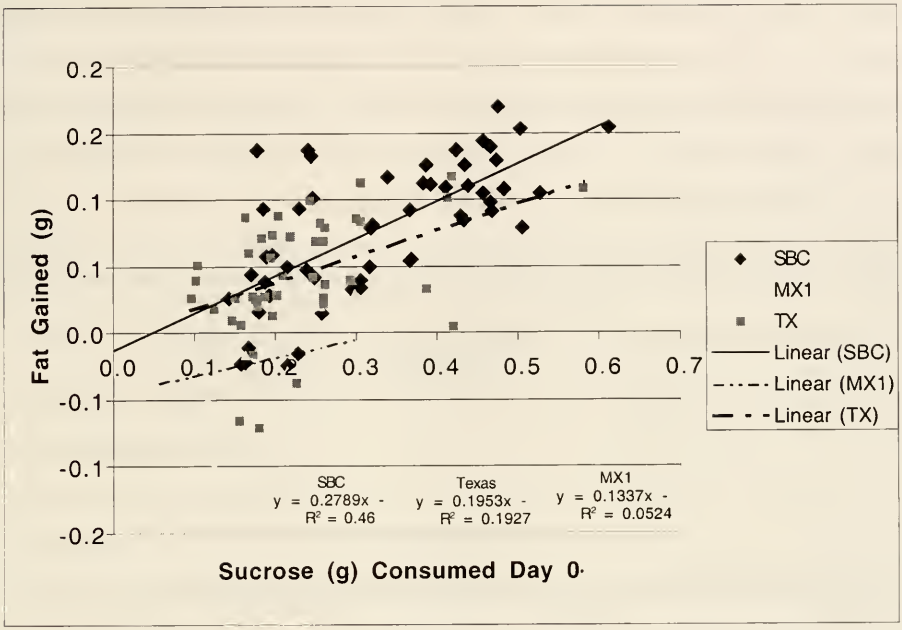


Table 19- Results From Linear Regression for Day 0 Butterflies, Fat Mass vs. Initial Wet Weight(X= initial wet weight and Y= fat gained) (N.S.=not significant, *<0.05, **<0.005 and ***<0.001)

GROUP	LINE	R	R^2	P-VALUE
SBC	Y= -0.84 + 0.265(X)	0.620	0.384	*
TX	Y= -0.133 + 0.509(X)	0.85	0.72	**
MX1	Y= -0.034 + 0.335(X)	0.74	0.54	***
MX2	Y= -0.002 + O.152 (X)	0.35	0.12	N.S.

Efficiency of Conversion of Sucrose to Fat

Using the estimates of fat gained or last, I determined that efficiency of converting sucrose to fat decreases from the SBC to TX to MX1 samples (Figure 20). For each sample the slope of the regression line represents the efficiency of the conversion between grams of sucrose consumed and change in fat. The SBC butterflies had an

efficiency of 28%, the TX butterflies had an efficiency of 20%, and the MX1 butterflies had an efficiency of 13%. A SBC butterfly consumed 0.25 grams of sucrose and gained 0.10 grams of fat, while a MX1 butterfly consumed 0.25 grams of sucrose and lost 0.046 grams of fat. There is a trend in the graph, however, there was no significant difference in the efficiency of the SBC, TX, and MX1 samples (Table 20). There was a significant difference in the change in fat over the feeding period for the samples (1-Factor ANOVA, $F_{2, 107} = 42.32$, $p<0.0001$). The SBC butterflies accumulated the most amount of fat, then the TX butterflies, then the MX1 butterflies (Figure 21).

Table 20- 2- Factor ANOVA Results for Efficiency (Sucrose to Fat) for Sweet Briar and Texas Fall Migrants and Early and Late Mexico Overwintering Butterflies (Table uses data from butterflies that were fed 20% or 45% sucrose solution that were killed on Day 7) (N.S.=not significant and *<0.05)

	DEGREES OF FREEDOM	F-VALUE	P-VALUE
SAMPLE	2	1.98	N.S.
SUCROSE CONCENTRATION	1	7.27	**
SAMPLE X SUCROSE CONCENTRATION	2	1.09	N.S.
RESIDUAL	140		

Discussion

Overview

The samples of butterflies had varying appetites, which resulted in differences in consumption and in whether fat was accumulated, maintained, or lost. The SBC migrants had the largest appetites, then the TX migrants, and there was no difference in the consumption of the MX1 and MX2 butterflies.

The SBC and TX butterflies responded to the laboratory conditions as hypothesized: they accumulated fat by drinking large amounts of sucrose solution. The hypothesis was based on the frequent observations of fall migrant butterflies nectaring as they fly south and the data that demonstrate that the fall migrants accumulate extensive lipid supplies prior to overwintering (Walford 1980, Brower 1995). The fact that they accumulated fat indicates that my methods are conducive to fat synthesis.

In contrast, even when unlimited food supplies were offered, the overwintering butterflies did not consume enough sucrose in the lab to accumulate fat. In fact, all of the overwintering butterflies that were fed lost fat over the duration of the experiment except the MX2 45% fed butterflies.

The efficiency data (Figure 20) imply that the conversion rate of sucrose to fat may decrease as the butterflies age. There might be differences in the fall migrants versus overwintering butterflies in the ability to metabolize sucrose or synthesize fat. Although differences were not significant, a trend exist that should be studied.

Energy Requirements for Spring Migrants

The fat masses of the Day 0 butterflies closely resemble patterns previously observed over the duration of the fall migration and overwintering period (Figure 1 and Figure 18). However, the means of my different samples were higher than the means for butterflies that were in similar parts of the fall migration and overwintering period from Walford (1980) and Brower (1995), but statistics were not used to determine if the differences were significant.

Late overwintering butterflies still have measurable stored levels of fat. The mean fat mass of my MX2 butterflies was approximately double the fat mass of freshly eclosed butterflies and triple that of summer breeding generations (Walford 1980, Brower 1995). This might imply that spring migrants have enough remaining fuel to provide them with energy to fly to milkweed sources and provide yolk for their eggs without accumulating additional lipid supplies. The females butterflies can also receive additional nutrient transfer from males during mating, allowing the females to migrate greater distances (Boggs and Gilbert 1979, Oberhauser 1992, Wells et al 1993). In addition, the spring remigrants may obtain additional nutrients to fuel their migration to larval food sources by feeding on nectar which can contain amino acids, proteins, lipids, antioxidants, organic acids, and various sugars: mainly, sucrose, glucose, and fructose (Boggs 1987, Leather 1995, Gilbert and Singer 1975, Baker and Baker, 1975, 1982, Romeis and Wackers 2000).

Ultimate and Proximate Questions

One ultimate question about the findings of this experiment is: Is the change in appetite of the migratory butterfly adaptive or is it a non-adaptive by-product result of aging or diapause? The major proximate question is: What mechanisms regulate meal sizes, fat synthesis, storage, and metabolism?

Natural Selection: Is the decrease in appetite adaptive?

High consumption in the fall can result in a fat butterfly that has plenty of lipid reserves to provide it with energy for the winter and remigration to the southern United States (Walford 1980, Brower 1995). The decrease in consumption observed in the overwintering butterflies under the laboratory conditions suggests that fat, healthy overwintering butterflies are not programmed to supplement their fat by nectaring. This may be an adaptive trait favored by natural selection because of the insufficient supply of nectar sources in the overwintering sites. Brower and Malcolm (1991) found that nectaring butterflies at the overwintering sites had lower fat masses than the inactive butterflies. This observation was supported by Alonso-Mejía et al (1997); nectaring butterflies in a Mexico overwintering site were found to have less than half as much fat as inactive butterflies clustered on trees in December, January, February, and March. Therefore, fat overwintering butterflies probably have enough fuel to provide them with energy for the winter period and, therefore, have no need for foraging. Natural selection probably prefers appetite changes because they can lead to increased survivorship. A side effect of diapause is a lowered metabolism, which may help play an integral role in the conservation of energy reserves (Sauer et al 1986).

The nectaring butterflies at the overwintering sites are similar to the summer generations of butterflies in their amount of fat, wet weight, and tattered wings (Van Hook 1993). The butterflies and other nectarivorous animals that inhabit the overwintering areas so greatly outnumber nectar availability that the cost of foraging for nectar is probably much greater than the benefit. Therefore, lean overwintering butterflies that are reduced to nectaring to meet daily metabolic needs are probably highly selected against.

The decreased appetite therefore, may be caused by aging or senescence of the overwintering butterflies. Further research needs to take place to determine if the ability to absorb and digest sucrose and synthesize fat diminishes with age. Because nectar supplies are insufficient in the overwintering areas the butterflies may lose their ability to accumulate new fat. The synthesis of digestive molecules may be costly, consequently the butterflies could benefit from losing their ability to digest sucrose when supplies are low and energy conservation play an integral role in the survivorship of the population. It is known that over the course of an insect's life history, its lipid content can fluctuate. These fluctuations result in a metabolic balance between the synthesis and utilization of lipids. The fluctuations are controlled by the direct or indirect actions of hormones and/or neurotransmitters of the endocrine system (Rochstein 1978).

Aging: Is the change in appetite non-adaptive?

It has been demonstrated that other Lepidoptera have decreased ability to accumulate fat as they age. Gunn and Gatehouse (1986) demonstrated that *Spodoptera exempta* (more commonly known as the African armyworm) was only able to acquire new fat reserves within the first day or two after eclosion for the duration of its adult life. Gunn and Gatehouse divided the subjects into different feeding groups and killed them off through time. The feeding groups consisted of moths that were starved, moths that were fed only water, and moths that were fed only a 10% sugar water solution. Lipid content of the moths after eclosion increased for the first 48 hours and then decreased, regardless of the feeding treatment. However, the moths that consumed the sugar water solution did not lose fat mass as rapidly as the moths that were in the other treatment groups. Monarchs might resemble the armyworms in having a 'cut-off point' in their maturation where they can no longer acquire new fat reserves. However, monarchs are different from the armyworms in that they are long as adults and that it is known that they are able to accumulate fat over a longer period of time.

Pullin (1986) performed a laboratory feeding experiment on adult feeding time, lipid accumulation, and overwintering in another butterfly, *Inachis io*. He found that the *Inachis io* accumulated fat from days 0 to 15. From days 16 to 30 fat mass of *Inachis io* decreased.

Mechanisms of Appetite Change

It is known that fall and overwintering monarchs are physiologically different from summer breeding populations. They have decreased levels of juvenile hormone (JH), a lipid hormone responsible for the acceleration of reproductive organ growth in males and females, and the acceleration of senescence, which provokes reproductive diapause and the migration south (Pam and Wyatt 1971, 1976, Barker 1976, Barker and Herman 1973, Herman 1975a, b, Johnson 1979). It is possible that low levels of this environmentally sensitive molecule may indirectly render the appetite of the migratory butterflies during the annual migratory cycle, as well as preventing adult females from futile reproduction in the fall in which offspring would be destined to death (Herman 1993).

Conservation

The results of this experiment have significant conservation implications. The late overwintering butterflies can no longer accumulate fat; therefore, fall nectar resources and the microclimate in the overwintering sites play an integral role in the survivorship and fecundity of the butterflies. Fall migrants accumulate the greatest amount of fat in Texas and other southern states. If land use modifications in these areas were to lower nectar

availability, this could cause monarch butterflies to begin the winter with smaller energy reserves. The use of broad spectrum herbicides is another growing concern because it kills nectar sources (Berger 1978) which in turn could also cause butterflies to arrive in the overwintering sites with less fat. The canopy of trees in the overwintering sites works as a "blanket" and an "umbrella," providing the butterflies with a more consistent environment. If the forest is thinned it experiences more extreme temperatures, winds and precipitation. Higher ambient temperatures are reached during the day, which raises the butterflies' metabolic rate, causing them to utilize their lipid reserves more quickly than the butterflies that inhabit the unthinned forests (Anderson and Brower 1996).

Hoth (1995) hypothesizes that thinning of the fir forests would allow growth of nectar sources, and, therefore, allow more monarchs to replenish their energy reserves, and in theory increase the number of butterflies that survive the winter. However, this research suggests that the overwintering butterflies are not programmed to nectar because even when the overwintering butterflies were given unlimited sucrose solution they lost fat. Hoth ignores the findings of Brower and Malcolm (1991) when they suggest that any changes to the overwintering sites that would cause the butterflies to increase their energy expenditure will in turn decrease the butterflies' chances of surviving the winter. Hoth believes that with this hypothesis, a "dogma" is born. He thinks that because he has observed "butterflies sitting on shrubs with their proboscides unrolled" that nectaring in the overwintering sites plays an integral role in the survivorship of the butterflies. Hoth overlooks the data that confirm that the feeding butterflies are unhealthy and have little or no fat; where as the inactive butterflies that exist in clusters had substantial energy reserves (Walford 1980, Brower and Malcolm 1991, Brower 1995, Alonso-Mejía et al 1997).

Future Research

The next step in further understanding the monarch butterfly and its appetite, ability to accumulate fat, and dependency on its fat reserves accumulated prior to overwintering is to examine the spring migrants to determine if they have increased consumption compared to overwintering butterflies and if they can accumulate fat to fuel migration to larval feeding groups and increase fecundity. If female monarch butterflies are capable of acquiring new fat reserves once they begin their remigration to the United States, some of their reliance on the fat acquired the previous fall is alleviated. If the females are incapable of gaining fat following the overwintering period, she will be totally dependent on the fat that she accumulated in the fall for migration and egg laying. Urquhart (1960) noted that the female rarely stops to nectar on flowers during the spring migration.

Conclusion

In conclusion, the appetite of female monarch butterflies varies over the course of the fall migration and overwintering period, which directly affects their ability to accumulate or maintain fat. The fall migrants are programmed to consume large amounts of sucrose, which is then turned into fat. The overwintering butterflies, on the other hand, are programmed not to eat. The data from this experiment imply that the preservation of the nectar supplies along the eastern migratory routes and protection of overwintering habitat of the monarch butterfly is greatly needed to prevent the extinction of this endangered biological phenomenon.

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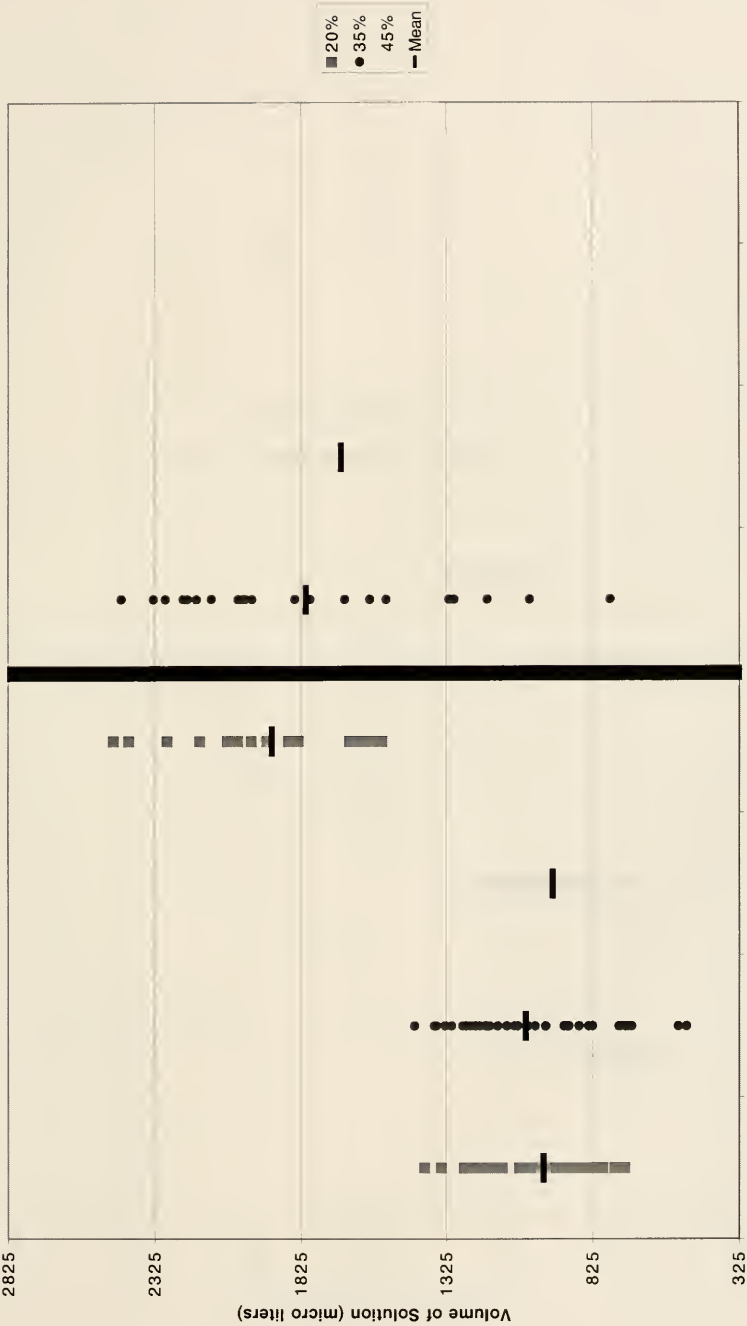


Figure 4- Sweet Briar- Total Volume of Sucrose Solution Consumed

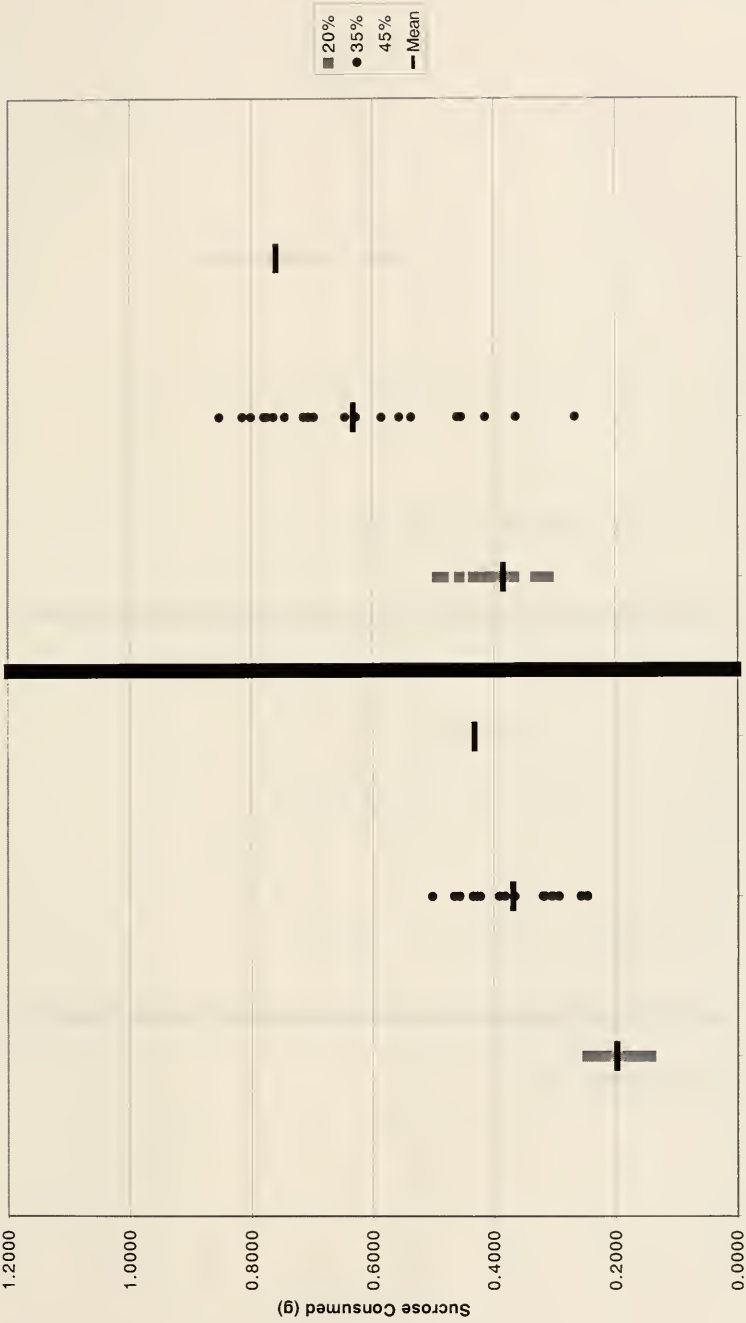


Figure 5- Sweet Briar Fall Migrants- Grams of Sucrose Consumed

Figure 6- Sweet Briar Fall Migrants- Fat Mass

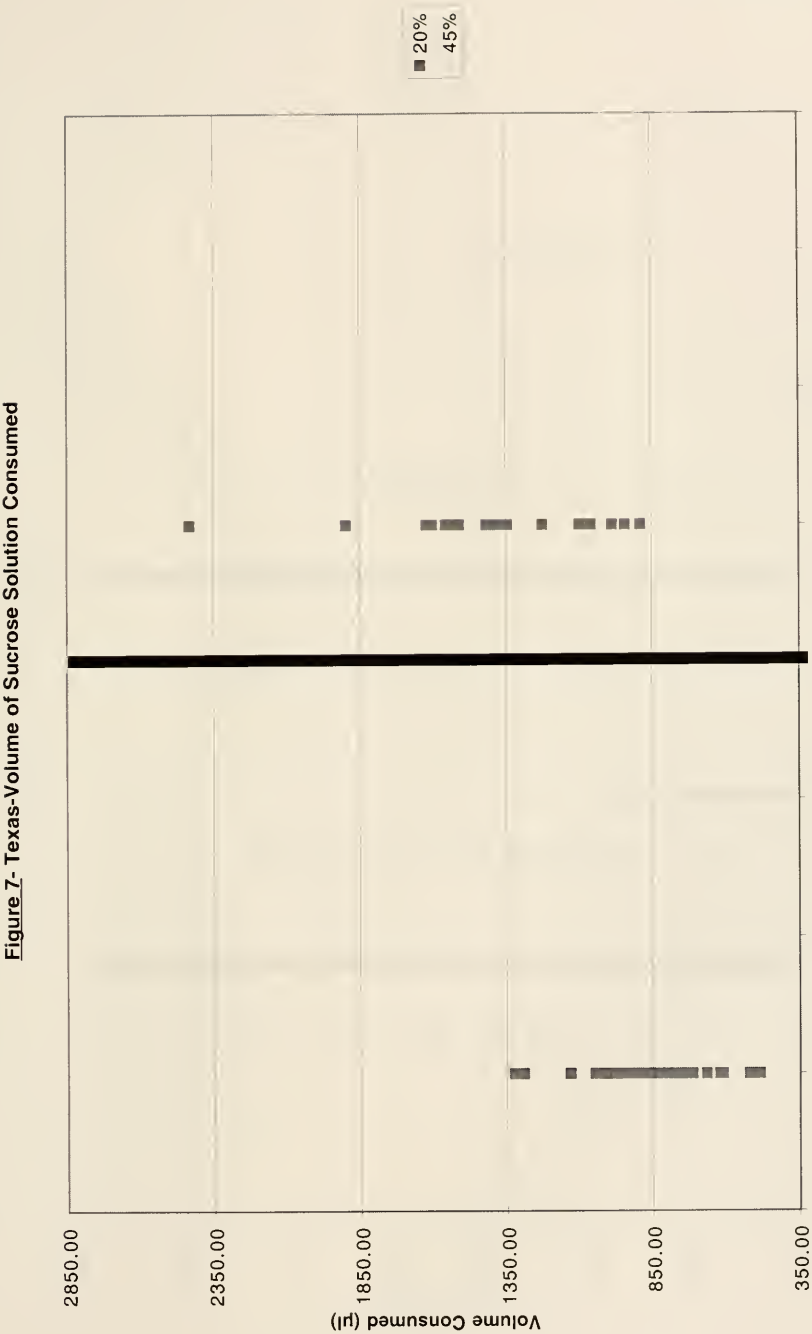


Figure 7- Texas-Volume of Sucrose Solution Consumed

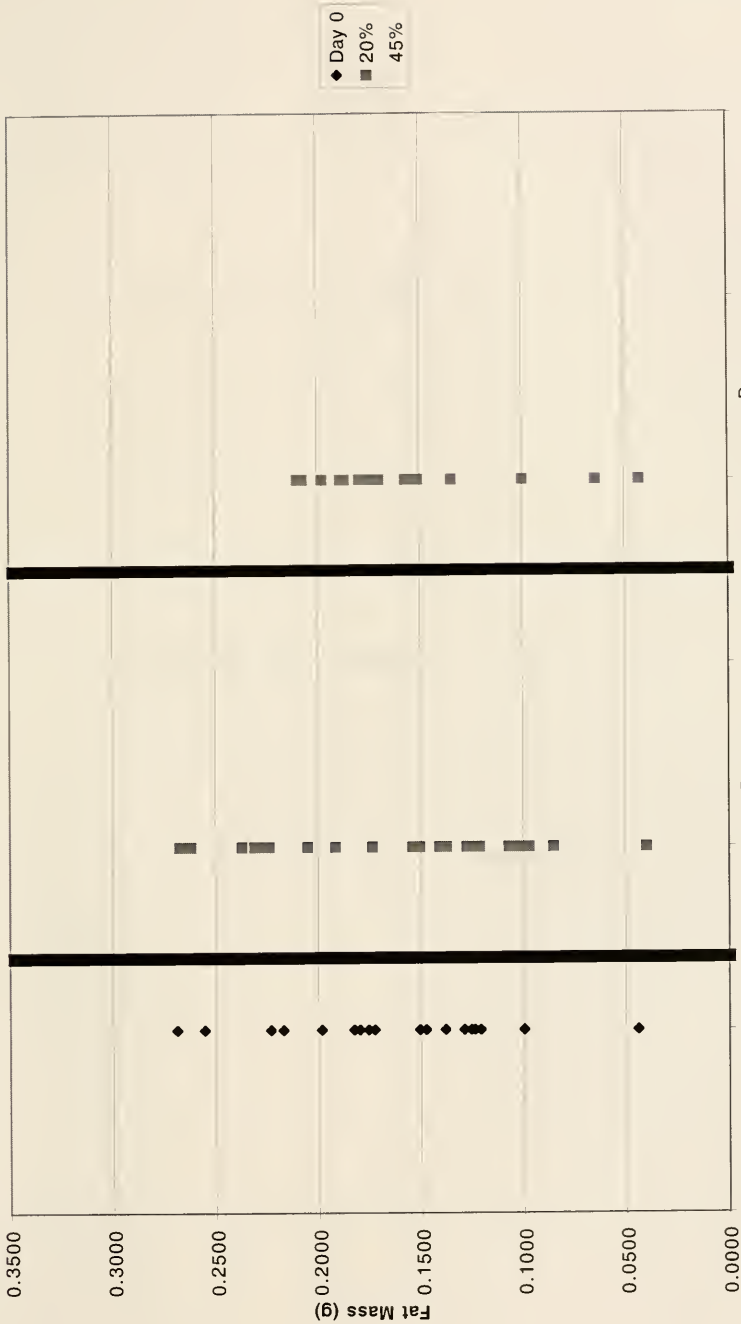


Figure 9-Texas- Fat Mass

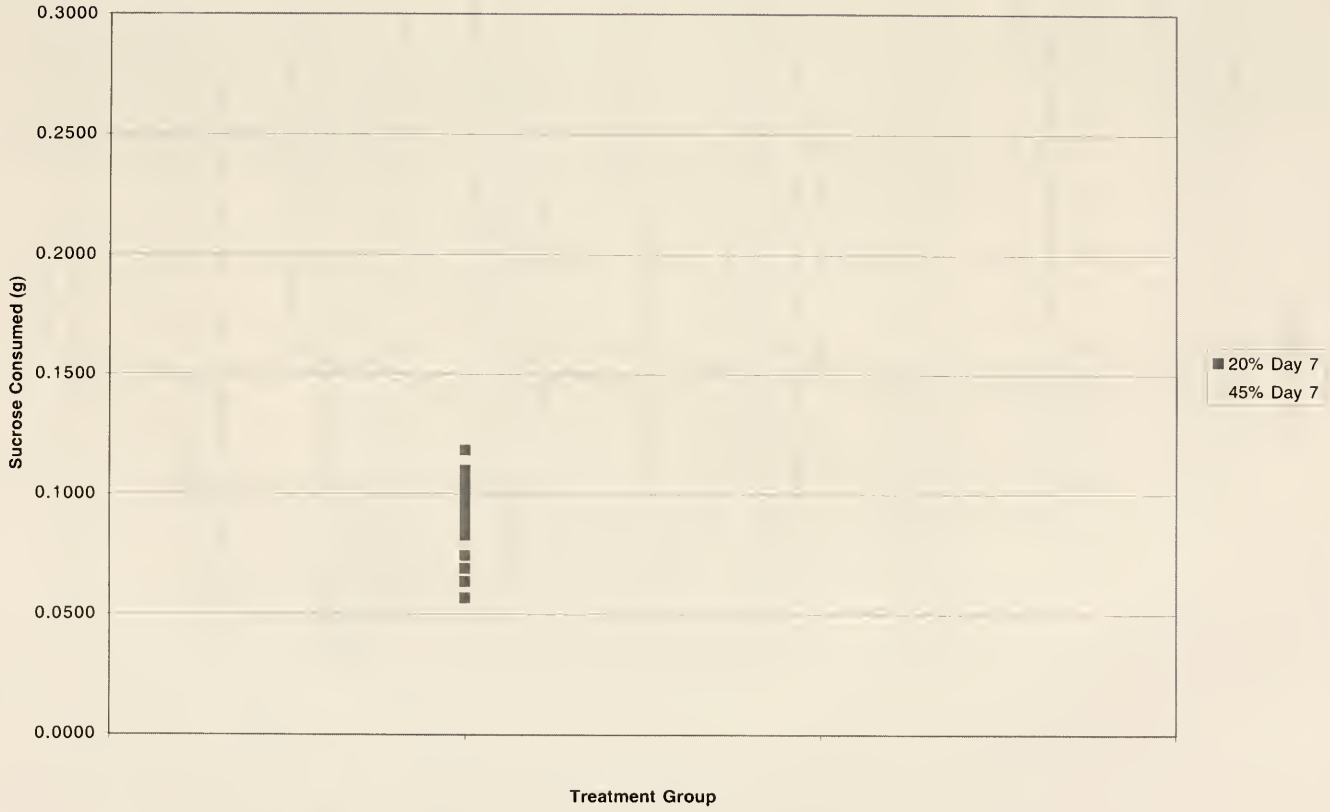
Figure 11-Early Mexico Overwintering- Grams of Sucrose Consumed

Figure 10- Early Mexico Overwintering- Total Volume of Sucrose Solution Consumed

Figure 12- Early Mexico Overwintering- Fat Mass

Figure 13- Late Mexico Overwintering- Volume of Sucrose Consumed

Figure 14- Late Mexico Overwintering- Grams of Sucrose Consumed



References

Alonso-Mejía, A. 1996. Biology and conservation of overwintering monarch butterflies in Mexico. Unpublished Graduate Dissertation, University of Florida.

Alonso-Mejía, A., L. P. Brower, E. Montesinos-Patio, and E. Rendon-Salinas. 1997. Use of lipid reserves by monarch butterflies overwintering in Mexico: implications for conservation. *Ecological Applications* **7(3)**: 934-947

Baker, H. G. and I. Baker. 1975. Studies of nectar-constitution and pollinator-plant coevolution. *Coevolution of Animals and Plants* (ed. by L. E. Gilbert and P. H. Raven), Pp. 100 –140. University of Texas Press, Austin, Texas.

Baker, H. G. and I. Baker. 1982. Chemical constituents of nectar in relation to pollination Mechanisms and phylogeny. *Biochemical Aspects of Evolutionary Biology* (ed. by M. H. Nitecki), Pp. 131 –171. University of Chicago Press, Chicago.

Beall, G. 1948. The fat content of a butterfly *Danuas plexippus* Linn, as affected by the migration. *Ecology* **29**: 80 –94.

Beenakkers, A. M. T., D. J. van der Horst, and W. J. A. van Marrewijik. 1981 Role of lipids in energy metabolism. Pages 53 –100 *in* R. G. H. Downer, editor. *Energy Metabolism in Insects*. Plenum Press, New York, New York, USA.

Berger, G. V. 1978. Agriculture for wildlife. Pp. 89 –120 in H.P. Brokaw (ed.) *Wildlife and America*. Council on Environmental Quality, U.S.A. Fish and Wildlife Service, Forest Service and National Oceanic and Atmospheric Administration, U.S.A. Fish and Wildlife Service, Forest Printing Office, Washington, D.C.

Boggs, C. L. 1987. Ecology of nectar and pollen feeding in Lepidoptera. *Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates* (ed by F. Slansky, Jr and J. G. Rodriguez), Pp. 369 –391. John Wiley and Sons, New York.

Boggs, C. L. 1988. Rates of nectar feeding in butterflies: effects of sex, size, age, and nectar concentration. *Functional Ecology* **2**: 289 –295.

Boggs, C. L. and L. E. Gilbert. 1979. Male contribution to egg production in butterflies: Evidence evidence for transfer of nutrients at mating. *Science*. **206**: 83 –84.

Brower, L. P. 1985. New Perspectives on the Migration Biology of the Monarch butterfly, *Danaus plexippus* L. *in* M. A. Rankin (eds.), *Migration: Mechanisms and Adaptive Significance* Pp. 748 –785. Austin, Texas: University of Texas

Brower, L. P. and S. B. Malcolm. 1991. Animal migrations: endangered phenomena. *American Zoologist* **31**: 265 –276.

Brower, L. P., G. Castilleja, A. Peralta, J. Lopez-Garcia, L. Bojorquez-Tapia, S. Diaz, D. Melgarejo, and M. Missrie. 2002. Quantitative changes in forest quality in principle overwintering area of the monarch butterfly in Mexico: 1971 to 1999. *Conservation Biolocy* **16**: 1 –15.

Brown, J. J. and G. M. Chippendale. 1974. Migration of the monarch butterfly, *Danaus plexippus*: energy sources. *Journal of Insect Physiology* **20**: 1117 –1130.

Calvert, W. H, and L. P. Brower. 1986. The location of monarch butterfly (*Danaus plexippus)* overwintering colonies in Mexico in relation to topography and climate. Journal of *Lepidopterist's Society* **35**: 216 –225.

Calvert, W. H., S. B. Malcolm, J. I. Glendinnind, L. P. Brower, M. P. Zalucki, T. V. Hook, J. B. Anderson, and L. C. Snook. 1989. Conservation of the monarch butterfly overwintering sites in Mexico. *Vida Sylvestre Neotropical* **2**: 38 –48.

Cenedella, R. J., 1971. The lipids of the female monarch butterfly, *Danaus Plexippus* during fall migration. *Insect Biochemistry*. **1**: 244- 247

Evans, H. A. 1984. *Insect Biology*. Adision-Wesley Publishing Company. Pp. 83

Gibo, D. L. and J. A. McCurdy. 1993. Lipid accumulation of migrating monarch butterflies (*Danaus plexippus L.*). *Canadian Journal of Zoology*. **71**: 76-82

Gilbert, L. E. and M.C. Singer. 1975. Butterfly ecology. *Annual Review of Ecology Systematics* **6**: 365 –397.

Gunn, A. and A. G. Gatehouse. 1986. The effect of adult feeding on lipid and protein reserves in African armyworm, *Spodoptera exempta*, moths before and during reproduction. *Physiological Entomology*. **11**: 423-431

Hoth, J. 1995. Mariposa monarca, mitos y otras relidades aladas. Revista Ciencias (Mexico) **37**: 19 –28.

James, D.G. 1984. Phenology of weight, moisture, and energy reserves of Australian monarch butterflies, *Danuas plexippus*. Ecological Entomology **9**: 421 –428.

James, D. G. 1993. Migration biology of the monarch butterfly in Australia. Pages 189 –200 *in* S. B. Malcolm and M. P. Zalucki, editors. Biology and Conservation of the monarch butterfly. Natural History Museum of Los Angeles County, Science Series 38.

Kerkut, G. A. and L. I. Gilbert. *Comprehensive Insect Physiology- Biochemistry and Pharmacology*. Peragamon Press. **11**: 29- 30

Kozhantshikov, I. W. 1938. Carbohydrate and fat metabolism in adult Lepidoptera. Bulletin of Entomological Research **29**: 103 –114.

Leather, S. R. 1995. Factors affecting feucundity, fertility, oviposition, and larviposition in insects. *Insect Reproduction* (ed. by S. R. Leather and J. Hardie, Pp. 143 –174. CRC Press, Bocca Raton, Florida.

Masters, A. R., S. B. Malcolm, and L. P. Brower. 1988. Thermoregulatory behavior and adaptions for overwintering in the monarch butterfly (*Danaus plexippus*) in Mexico. Ecology **69**: 458 -467

May, Peter G. 1985. A simple method for measuring nectar extraction rates in butterflies. *Journal of the Lepidopterises' Society*. **39**(1): 53-55

Oberhauser, K. S. 1992. Rate of ejaculate breakdown and intermating intervals in monarch butterflies. *Behavioral Ecology Sociobilogy*. **31**: 367 –373.

Picciano, M. F. and H. A. Guthrie. 1998. *Human Nutrition*. Mosby, (ed. 4) Pp. 111-151

Pullin. A. S. 1986. Adult feeding time, lipid accumulation, overwintering in *Aglais uritcae* and *Inachis io* (Lepidoptera: Nymphaldae). *The Zoological Society of London* **211**: 631 –641.

Rockstein, M. 1978. *Insect Biochemistry*. Pp. 84 –88. Academic Press, INC, New York.

Romeis, J. and F. L. Wackers. 2000. Feeding responses by female *Prieris brassicae* butterflies to carbohydrates and amino acids. *Physiological Entomology* **25:** 247 –253.

Rzedowski, J. 1983. Vegetation of Mexico, (Ed.) Limusa, Mexico, D.F., Mexico

Sauer, K.P., H. Spieth, C. Gruner. 1986. Adaptive significance of genetic variability of photoperiodism in Mecoptera and Lepidoptera. *in* F. Taylor and R. Karban, editors. *The Evolution of Insect Life Cycles*. Springer-Verlg, New York

Snook, L. 1993. Conservation of the monarch butterfly reserve in Mexico: focus on the forest *in* S. B. Malcolm and M. P. Zalucki, editors. Biology and Conservation of the monarch butterfly. Natural History Museum of Los Angeles County, Science Series 38.

Turunen, S. and G. M. Chippendale. 1980. Lipid Transport in the migrating monarch butterfly *Danuas p. plexippus*. Experientia **37**: 266-268

Tuskes, P. M., and L. P. Brower. 1978. Overwintering ecology of the monarch butterfly, *Danaus plexippus* L., in California. Ecological Entomology **16**: 175 –184.

Urquhart, Frederick A. 1960. *The Monarch Butterfly* Pp. 77 –94 and 252 –321. University of Toronto Press: 300- 310

Urquhart, Frederick A. 1960. *The Monarch Butterfly* Pp. 319-321. University of Toronto Press: 300- 310

Urquhart, F. A., and N. R. Urquhart. 1978. Autumnal migration routes of the eastern population Of monarch butterfly (*Danaus p. plexippus*; Danaidae: Lepidoptera) in the North America To the overwintering site in the neovolcanic plateau of Mexico. Canadian Journal of Zoology **56**: 1754 –1764.

Walford, P. 1980. Lipids in the life cycle of the monarch butterfly, *Danaus plexippus*. Unpublished honors thesis, Dept of Biology, Amherst College, Amherst, MA.

Wells, H., P. H. Wells, and S. Rogers. 1993. Is multiple mating an adaptive feature of monarch butterfly winter aggregations? *in* S. B. Malcolm and M. P. Zalucki, editors. Biology and Conservation of the monarch butterfly. Natural History Museum of Los Angeles County, Science Series 38.

Van Hook, T. 1993. Nonrandom mating in monarch butterflies overwintering in Mexico. Pp: 49 –60 *in* S. B. Malcolm and M. P. Zalucki, editors. Biology and conservation of the monarch butterfly. Natural History Museum of Los Angeles County, Science Series 38.

Weast, Robert C. Shelby, Samuel M. 1967. *Handbook of Chemistry and Physics*. The Chemical Rubber Company: E-36.

Wigglesworth, V. B. 1972. *The Principles of Insect Physiology*. Chapman and Hall, London.

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